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# Synthesis and Biological Activities of Ferulic Acid–Amino Acid Derivatives

Zhuang MIAO, Hiroshi KAYAHARA,* and Koji TADASA*

*The United Graduate School of Agricultural Science, Gifu University, 1–1 Yanagido, Gifushi, Gifu 501–11, Japan*
** Division of Bio-organic Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Shinshu University, 8304 Minamiminowamura, Kamiinagun, Nagano 399–45, Japan*
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**Three series of ferulic acid derivatives (feruloylaminoacid benzyl ester or methyl ester, feruloylaminoacid, and 4-*O*-[*N*-(carbobenzyloxy)-aminoacyl]ferulic acid) were synthesized newly, and their superoxide dismutase (SOD)-like, platelet aggregation (PA)-inhibitory, tyrosinase-inhibitory, and angiotensin converting enzyme (ACE)-inhibitory activities were evaluated. 4-*O*-[*N*-(Carbobenzyloxy)isoleucyl]ferulic acid (20) and 4-*O*-[*N*-(carbobenzyloxy)prolyl]ferulic acid (21) showed potent PA-inhibitory activities and tyrosinase-inhibitory activities, while they also had SOD-like activities at the same level as ferulic acid.**

**Key words:** ferulic acid–amino acid derivatives; superoxide radical; platelet aggregation; tyrosinase; angiotensin converting enzyme

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a ubiquitous plant constituent that arises from the metabolism of phenylalanine and tyrosine. It occurs primarily in seeds and leaves both in its free form and covalently linked to lignin and other biopolymers. Owing to the presence of a phenolic nucleus having a conjugated side chain, it readily forms a resonance-stabilized phenoxy radical, which accounts for its potent antioxidant activity.[1] UV absorption by ferulic acid catalyzes stable phenoxy radical formation and thereby potentiates its ability to terminate free radical chain reactions.[2] It was reported that sodium ferulate inhibited the production of thromboxane $A_2$ ($TXA_2$) powerfully but had no influence on the production of prostaglandin $I_2$ ($PGI_2$).[3] This means that sodium ferulate may inhibit platelet aggregation by disturbing the arachidonic acid cascade. It was also reported that γ-oryzanol, a derivative of ferulic acid, had strong hypocholesterolemic and antiatherogenic properties.[4,5] Considering the safety of using ferulic acid derivatives in functional foods or cosmetics, the properties of ferulic acid–amino acid derivatives were thought to be interesting research targets. However, no report on the biological activities of either natural or synthetic ferulic acid–amino acid derivatives has been published. In this paper, we report the evaluation of the biological activities (SOD-like, PA-inhibitory, tyrosinase-inhibitory, and ACE-inhibitory activities) of some ferulic acid–amino acid derivatives.

Three series including 22 kinds of compounds were synthesized (Fig. 1). Benzyl esters or a methyl ester of feruloylamino acids were synthesized by the condensation of an amino group of a *C*-terminal protected amino acid with the carboxyl group of ferulic acid by the DCC procedure.[6] Feruloylamino acids were difficult to be obtained by catalytic hydrogenation of their benzyl esters because the final products became too messy to be purified owing to some side reactions, possibly caused by the active phenolic hydroxyl group of ferulic acid. The DSC procedure,[7,8] with moderate reaction conditions, was thus used in the condensation

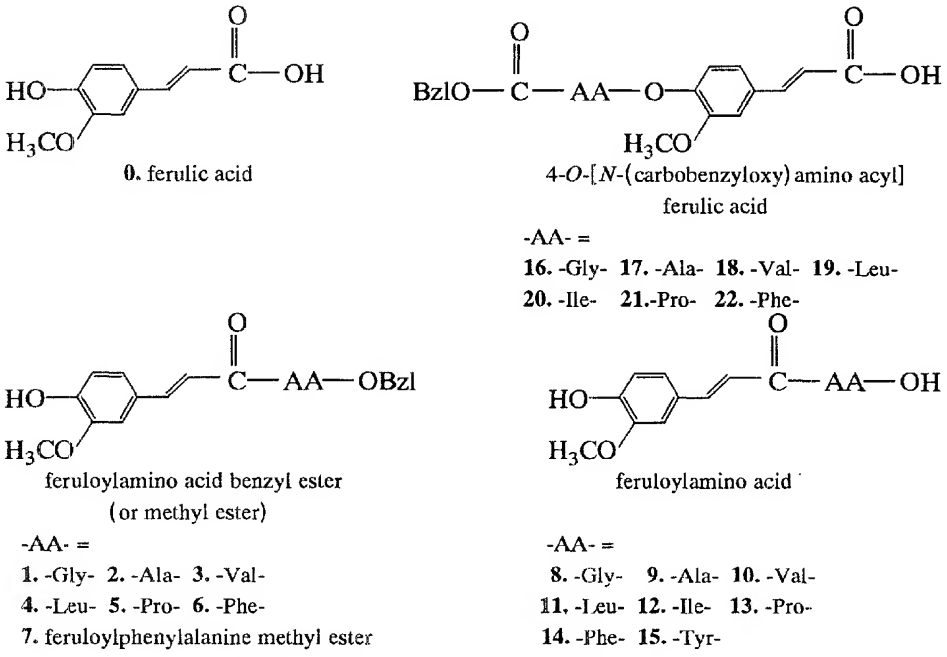


**Fig. 1.** Ferulic Acid–Amino Acid Derivatives

*Abbreviations*: SOD, superoxide dismutase; PA, platelet aggregation; ACE, angiotensin converting enzyme; DCC, dicyclohexylcarbodiimide; DSC, disuccinimidyl carbonate; Cyt. *c*, cytochrome *c*.

**Table** Biological Activities of the Ferulic Acid–Amino Acid Derivatives

| Compounds | SO-scavenging activity[a] (%) | PA-inhibitory activity[b] | | | T-inhibitory activity[c] (%) |
|---|---|---|---|---|---|
| | | ADP | | Collagen | |
| | | Inb. (%) | Dis. (%) | Inb. (%) | |
| Ferulic acid | 54.32±1.5 | 72.7±1.2 | 0.0 | 80.4±1.5 | 16.1±1.2 |
| FA–AA–OBzl | | | | | |
| **4** | — | — | — | — | 76.2±3.0 |
| **6** | — | — | — | — | 87.8±3.2 |
| FA–AA–OH | | | | | |
| **8** | 45.30±3.2 | 47.7±1.6 | 0.0 | 51.3±1.8 | 1.2±1.5 |
| **15** | 43.42±2.8 | 10.5±2.2 | 0.0 | 30.4±1.5 | 22.8±2.7 |
| Z–AA–FA–OH | | | | | |
| **16** | 54.51±3.5 | 83.3±1.5 | 9.3 | 83.6±2.6 | 26.0±1.6 |
| **20** | 32.52±3.0 | 71.3±2.8 | 23.1 | 76.6±3.0 | 25.1±2.1 |
| **21** | 44.36±3.2 | 70.0±1.8 | 25.0 | 54.3±2.2 | 32.8±1 4 |

FA–AA–OBzl, FA–AA–OH, and Z–AA–FA–OH are the abbreviations of feruloylamino acid benzyl ester, feruloylamino acid, and 4-*O*-[*N*-(carbobenzyloxy)aminoacyl]ferulic acid, respectively; —, no data.

[a] SO-scavenging activity was detected by the cytochrome *c* method,[9] and the final concentration of the test compound was adjusted to 1.5 mM.

[b] PA-inhibitory activity was detected with a PA tracer[10] ([NBS] Hema Tracer 601), platelets of dog blood were used, and the final concentration of the test compound was adjusted to 1.5 mM (containing an equimolar sodium sulfate).

[c] Tyrosinase-inhibitory activity was detected with the method which was reported in our previous paper,[11] and the addition concentration of the compound was 0.4 mM.

to produce the feruloylamino acids. 4-*O*-[*N*-(Carbobenzyloxy)-aminoacyl]ferulic acids were also synthesized by condensation of an *N*-terminal protected amino acid with the carboxyl group of ferulic acid using the DSC procedure. The chemical structures of all compounds synthesized above were confirmed by $^{1}$H- and $^{13}$C-NMR spectra (Bruker DRX-500 and AC-250 spectrometer), and high-resolution mass spectra (JEOL JMS-DX303HF).

Since most of the derivatives in the same group had biological activities at the same level, only the results of representative ones in each group are listed in the Table. The 4-*O*-[*N*-(carbobenzyloxy)aminoacyl]ferulic acid group was tested for tyrosinase inhibition only.

Most of the derivatives in both feruloylamino acid and 4-*O*-[*N*-(carbobenzyloxy)aminoacyl]ferulic acid groups, such as **8**, **15**, **20**, and **21**, represented a little bit weaker SO-scavenging activity than ferulic acid except for **16**, **17**, and **22** which had the activity at the same level as ferulic acid. In the mechanism similar to that of ferulic acid, feruloylamino acids may form a stable phenoxy radical by abstracting an electron from superoxide because of the presence of a phenolic nucleus and an extended side chain conjugation. However, the fact that 4-*O*-[*N*-(carbobenzyloxy)aminoacyl]ferulic acids also had the activity despite the absence of the active phenolic hydroxyl group suggests another mechanism. Because of the planarity of the entire molecule, ferulic acid and the feruloylamino acids are thought to be unlikely to form metal complex compounds, indicating that they owe most of their activity by reacting with superoxide. However, in the case of 4-*O*-[*N*-(carbobenzyloxy)-aminoacyl]ferulic acids, the change of the spatial structure by introduction of an amino acid at 4-OH may cause the formation of a metal complex compound to work as a superoxide dismutase-like compound.

Benzyl esters or a methyl ester of feruloylamino acid and 4-*O*-[*N*-(carbobenzyloxy)aminoacyl]ferulic acids inhibited tyrosinase activity more strongly than ferulic acid, but feruloylamino acids showed no obvious inhibition except for **14** and **15**. These results suggest that the derivatives inhibited tyrosinase activity competitively by the *N*-benzyl group, benzyl or phenolic group on the side chains of the amino acids.

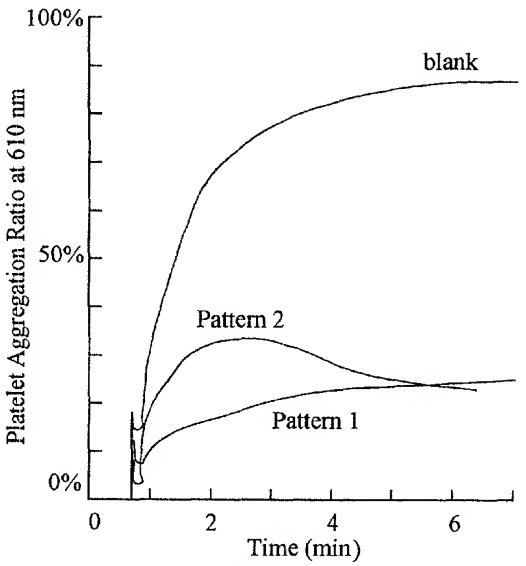


**Fig. 2.** Concentration-response Curves of Platelet Aggregation.

In the assay system, ADP may cause reversible aggregation. The fact that ADP-caused aggregation was dissolved once again was described as dissociation. The concentration-response curve of collagen-induced platelet aggregation was detected in pattern 1. The concentration-response curve of ADP-induced platelet aggregation was detected in pattern 1 (without dissociation) or pattern 2 (with dissociation).

Platelet aggregation, one of the repairing mechanisms for blood vessel injury, relates to some diseases such as thrombosis. Developing a compound capable of inhibiting platelet aggregation may provide a therapeutic tool for these diseases. From the results of this study (Fig. 2), the PA-inhibitory activity of feruloylamino acids were lower than that of ferulic acid against both ADP- and collagen-induced aggregations, and no dissociation was detected. In the case of 4-*O*-[*N*-(carbobenzyloxy)amino-acyl]ferulic acids, the inhibition against both ADP- and collagen-induced aggregations was kept on the same level as that by ferulic acid, and strong dissociations against ADP-induced

aggregation were detected with **20** and **21**. In other words, **20** and **21** may not only prevent thrombosis but also dissolve thrombosis.

In our laboratory, we have found that feruloylphenylalanylalanylproline had a strong ACE-inhibitory activity ($IC_{50}$ value = 1.5 $\mu$M),[12,13] but no obvious inhibition was detected in any of the ferulic acid–amino acid derivatives in this study.

In summary, 4-*O*-[*N*-(carbobenzyloxy)aminoacyl]ferulic acids are considered to scavenge superoxide at the same level as ferulic acid and have stronger tyrosinase-inhibitory activity than does ferulic acid. Since they are more hydrophobic compounds than ferulic acid, they may be effective as cosmetic ingredients. 4-*O*-[*N*-(Carbobenzyloxy)isoleucyl]ferulic acid and 4-*O*-[*N*-(carbobenzyloxy)prolyl]ferulic acid are more effective to inhibit platelet aggregation than ferulic acid.

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